Post-transplant T cell deficiency (especially in adults) is related to impaired thymus-dependent lymphopoiesis. However, others and we have previously found that peripheral T cell apoptosis in recipients of allogeneic bone marrow transplantation (allo BMT) is increased. We used murine allo BMT models to analyze peripheral T cell apoptosis in three different T cell populations after allo BMT: T cells derived from (a) donor bone marrow (BM) precursors (de novo generated), (b) infused mature donor T cells in the allograft (alloreactive and non-alloreactive), and (c) residual host T cells. In the first experiments, we found an increased percentage of apoptotic (Annexin-V+) T cells in both donor derived de novo generated CD4+ and CD8+ T cells at day 28 after transplant in recipients of a T cell depleted (TCD) BMT without GVHD. This was associated with decreased levels of intracellular Bcl-2 in de novo generated T cells from both young and old recipients of an allo TCD BMT. The increased percentages of apoptotic peripheral T cells and down regulation of Bcl-2 levels were more prominent in the older recipients. In recipients with GVHD, we found a severe loss of thymic cellularity, low numbers of BM derived de novo generated T cells with a high fraction of apoptotic cells and very low Bcl-2 expression and increased caspase 8 and 9 activation, Conversely, alloreactive T cells (derived from the infused mature donor T cells in the allograft) showed upregulation of Fas expression, higher Bcl-2 levels, less caspase activity and finally, lower numbers of apoptotic T cells compared to BM derived T cells. To determine the effects of Bcl-2 and Fas on T cell reconstitution in allo BMT recipients, we transplanted donor allografts consisting of BM from Bcl-2 overexpressing transgenic mice (Bcl-2 Tg) or BM from Fas-deficient lpr mice with or without wild type congenic T cells (B6.Ly5.1) into lethally irradiated recipients. Bcl-2 Tg as well as lpr BM rescued post-transplant thymic cellularity and distribution and increased CD4+ and CD8+ T cell numbers in the periphery, however no change in the fraction of apoptotic cells was observed. In contrast, post transplant administration of Interleukin-7 and Interleukin-15 reduced the number of apoptotic T cells in the periphery.

We conclude that peripheral T cell apoptosis is an important factor in the delay of post transplant T cell reconstitution especially in older recipients of allo BMT and in recipients with GVHD.

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IL-15 AS A POTENTIAL REGULATOR OF PERIPHERAL NK AND CD8+ T CELL HOMEOSTASIS

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Interleukin-15 (IL-15) has been demonstrated to play a critical role in the proliferation, differentiation, and survival of natural killer (NK) and CD8+ memory T cell populations. Because NK and memory CD8+ T cells recover rapidly following allogeneic transplantation, we investigated the role of IL-15 as a potential homeostatic regulator of these two cell types. 39 patients undergoing allogeneic transplant for hematological and non-hematological malignancies were assessed for plasma IL-15 levels by ELISA and IL-15 receptor expression by flow cytometry. The median plasma IL-15 level from healthy donors was 0.01 pg/ml (range 0.01-.12 pg/ml). Pretreatment median levels of IL-15 in patient plasma were 0.85 pg/ml (range 0.01-9.7); median NK levels were 105 cells/microL (range 5-573) and CD8+ levels were 327 cells/microL (range 19-1490). IL-15 levels increased through successive rounds of cytoreductive chemotherapy. Following a high dose fludarabine/cytoxan conditioning regimen preceding transplant, IL-15 levels were on average increased by 100 fold from pretreatment levels to a median of 44 pg/ml (range 25-401) ($P < 0.0001$). NK and CD8+ T cell levels at this point were reduced to a median level of 0 cells/microL (range 0-23). Concurrent with the rapid recovery of NK (median 223 cells/microL, range 0-816) and CD8+ (median 252 cells/microL, range 11-897) T cells at 2 weeks, IL-15 levels in the peripheral blood decreased to median levels of 8.7 pg/ml (range 0.9-160). IL-15 levels continued to fall concurrent with recovery of cell populations. Each of the 39 patients, regardless of patient age (adult or pediatric) or graft composition (CD34 selected versus T-replete) demonstrated the same spike in plasma IL-15 levels on the day of transplant. Consistent with a key role for IL-15, we found that the IL-2/IL-15 receptor beta was expressed at an elevated level on all NK cells and was increased on CD8+ T cells in the post transplant period, whereas the IL-2 receptor alpha chain was expressed at a low level in these populations. The rise in IL-15 levels may be due to reduced consumption by therapy diminished populations of lymphocytes or increased production in response to inflammatory cytokines triggered by the transplant regimen. In either case, these data support the possibility that IL-15 serves as a critical homeostatic cytokine post transplant, stimulating the initial rapid generation of NK cells and the expansion of NK and memory CD8+ T cell populations.

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T CELL REPERTOIRE COMPLEXITY IS CONSERVED AFTER L-LEUCYL-L-LEUCINE METHYL ESTER (LLME) TREATMENT OF DONOR LYMPHOCYTE INFUSIONS

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Delayed and/or incomplete T cell reconstitution following allogeneic hematopoietic stem cell transplantation (HSCT) is a major risk factor for patient mortality. One approach to rectify this problem is to administer immunocompetent T cells in the form of delayed donor lymphocyte infusions (DLI). However, the development of graft-versus-host disease (GVHD) is a potential complication of this procedure. We previously found in P → F1 haploidentical murine models that the ex-vivo treatment of donor lymphocytes with L-leucyl-L-leucine methyl ester (LLME) can prevent the onset of GVHD after DLI (BBMT 8:303,2002). LLME is a lysosomotropic agent that preferentially acts upon cells containing dipeptidyl peptidase I enzymes (DPPI) in their granules. The overall effect is to induce cell death of most NK cells, monocytes, granulocytes, the majority of CD8+ T cells, and a fraction of CD4+ T cells. Our previous preclinical studies have formed the basis of an ongoing phase I clinical trial in which patients received LLME-treated DLI from their original donor in an attempt to accelerate T cell reconstitution. In order to understand how this treatment strategy might impact upon the complexity of the DLI T cell repertoire, we used TCR Vβ spectratype analysis to evaluate the DLI product pre and post-LLME treatment. Peripheral blood T cells obtained from the donors at the time of DLI, prior to LLME treatment, served as the baseline spectratype for Vβ complexity. The repertoire complexity of each LLME-treated DLI product was determined by comparison to its untreated sample. The results of the spectratype analysis indicated that the LLME-treated DLI product exhibited CDR3-size distribution complexities similar to its untreated donor sample. In addition, comparisons of the CD4+ T cell repertoire from the donor before LLME treatment to that of the patient post DLI demonstrated equal complexity for 92% of the resolvable Vβ families. Lastly, the in vitro proliferative capacity of an LLME-treated product in response to third party stimulation in a one-way mixed lymphocyte reaction was comparable to the untreated product. Since it appears that LLME treatment of DLI does not adversely impact the complexity of the T cell repertoire or its functional capacity, this transplant strategy is likely to afford advantages with regard to mounting responses to opportunistic infections and preventing leukemic relapse.

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REDUCING PRETRANSPLANT CAMPATH-1H DOSE DOES NOT PREVENT EARLY CMV REACTIVATION POST ALLOGENEIC STEM CELL TRANSPLANTATION

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